

Application No.: 10/726,224

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AMENDMENTS TO THE CLAIMS

1. (Currently Amended): An assay for the presence or amount of an analyte in a sample comprising

a) combining in solution the sample to be assayed for the presence or amount of analyte; a ligand that binds the analyte in solution; a first substrate, a second substrate, and a scavenger compound; wherein

the analyte has a first enzymatic activity that acts on said first substrate to produce a colorless first product;

the ligand is directly or indirectly bound to an enzyme with a second enzymatic activity that acts on the second substrate to produce a colorless second product;

the scavenger compound is a scavenger for the first product or the second product;

the first product and the second product chemically combine to produce a colored reaction product; and,

the first enzymatic activity is a hydrolase activity or an oxidase activity and the second enzymatic activity is a hydrolase activity if the first enzymatic activity is an oxidase activity and is an oxidase activity if the first enzymatic activity is a hydrolase activity;

b) detecting the production of the colored reaction product; and

c) relating the production of the colored reaction product with the presence or amount of analyte ~~or amount~~ in the solution.

2. (Original): The method of claim 1 wherein the first enzymatic activity is a hydrolase activity.

3. (Original): The method of claim 1 wherein the first enzymatic activity is an oxidase activity.

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4. (Original): The method of claim 1 wherein the scavenger is 3-amino-1-(2,4,6-trichlorophenyl)-2-pyrazolin-5-one or acetoacetamide.
5. (Original): The method of claim 4 wherein the scavenger is 3-amino-1-(2,4,6-trichlorophenyl)-2-pyrazolin-5-one.
6. (Original): The method of claim 4 wherein the scavenger is acetoacetamide.
7. (Original): The method of claim 1 wherein said ligand is an antibody or a lectin.
8. (Original): The method of claim 7 wherein said ligand is an antibody.
9. (Original): The method of claim 1 wherein whichever of the first and second substrate is the substrate of the hydrolase activity is a compound that comprises a benzene ring or naphthalene structure with one active hydroxyl group.
10. (Original): The method of claim 9 wherein the substrate is 1-naphthol phosphate or phenyl phosphate.
11. (Previously Presented): The method of claim 1 wherein the whichever of the first and second substrate is substrate of the oxidase activity is selected from the group consisting of N,N-dimethyl paraphenylene diamine; N,N-diethyl paraphenylene diamine; N-phenyl paraphenylene diamine; N'-ethyl-N'-ethyl-(2-methylsulfonamidoethyl)-2-methyl-1,4-phenylene diamine; 4 amino antipyrine; and N,N-dimethylamino benzidine.
12. (Original): The method of claim 11 wherein the substrate is N'-ethyl-N'-ethyl-(2-methylsulfonamidoethyl)-2-methyl-1,4-phenylenediamine.

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13. (Original): The method of claim 1 wherein the hydrolase activity is selected from the group consisting of a phosphatase, an esterase, a galactosidase, a lipase, a glucuronidase, an amidase, a peptidase, and a sulphatase.

14. (Original): The method of claim 3 wherein the oxidase activity is a pseudoperoxidase activity.

15. (Original): The method of claim 14 wherein the analyte is glycated hemoglobin.

16. (Original): The method of claim 15 wherein the solution comprises non glycated hemoglobin and the glycated portion of hemoglobin to be compared to total hemoglobin.

17. (Original): The method of claim 15 wherein the ligand is an organic boronic acid compound directly or indirectly conjugated to a hydrolase.

18. (Original): The method of claim 1 wherein whichever of the first and second substrate is the substrate of the oxidase activity is selected from the group N,N-dimethyl paraphenylene diamine; N,N-diethyl paraphenylene diamine; N-phenyl paraphenylene diamine; N'-ethyl-N'-ethyl-(2-methylsulfonamidoethyl)-2-methyl-1,4-phenylene diamine; 4 amino antipyrène; and N,N-dimethylamino benzidine,

whichever of the first and second substrate is the substrate of the hydrolase activity is naphthyl phosphate or phenyl phosphate, and

the scavenger is 3-amino-1-(2,4,6-trichlorophenyl)-2-pyrazolin-5-one or acetoacetamide.

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19. (Original): The method of claim 18 wherein the substrate of the hydrolase activity is naphthyl phosphate or phenyl phosphate and the substrate of the oxidase activity is N'-ethyl-N'-ethyl-(2-methylsulfonamidoethyl)-2-methyl-1,4-phenylenediamine.

20. (New): The method of claim 9, wherein the one active hydroxyl, further comprises a blocking group.

21. (New): The method of claim 20, wherein the blocking group is an organic compound.

22. (New): The method of claim 21, wherein the blocking group is selected from the group consisting of Ac-Tyr-Val-Ala-Asp--, Ac-Asp-Glu-Val-Asp--, Ac-Leu-Glu-Val-Asp--, Ac-Trp-Glu-His-Asp--, Ac-Val-Glu-Ile-Asp-- and Ac-Leu-Glu-His-Asp--.

23. (New): The method of claim 21, wherein the blocking group is selected from the group consisting of Glucose-alpha--, Glucose-beta--, Galactose-alpha--, Galactose-beta--, N-Acetyl glucosamine-- and Glucuronic acid--.

24. (New): The method of claim 21, wherein the blocking group is selected from the group consisting of HO-Darg-Gln-Gly-Ala-Ill-Gly-Gln-Pro--, Pyr-Pro-Val-- and Benzoyl DL Arginine--.

25. (New): The method of claim 21, wherein the blocking group is selected from the group consisting of acetate--, chloroacetate-- and buyrate--.

26. (New): The method of claim 20, wherein the blocking group is an inorganic compound.

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27. (New): The method of claim 26, wherein the blocking group is selected from the group consisting of HO(OO)PO-- and HO(OO)SO--.

28. (New): The method of claim 1 wherein whichever of the first and second substrate is the substrate of the hydrolase activity is a compound comprising the general formula R-B, wherein R is a colored coupler compound having an active hydroxyl group and B is an organic or inorganic blocking group specific for the hydrolase.

29. (New): The method of claim 28, wherein R is selected from the group consisting of Hydroxybenzine (Phenol), 4-chloro-1-Hydroxybenzine (4-Chlorophenol), 2-chloro-1-hydroxybenzine (2-chlorophenol), Aminobenzine (anilin), 4-chloro-1-aminobenzine (4-chloro-1-aminobenzine), 2-chloro-1-aminobenzine (2-chloro-1-aminobenzine), 1-Naphthol, 2-Naphthol, 4-chloro-1-naphthol, 8-hydroxy-naphthalene-1-sulphonic acid, 4-nitrophenol, 2-chloro-4-nitro-phenol, 2-chloro-4-nitro-1 naphthol and 5-nitro-8-hydroxy-naphthalene-1 sulphonic acid.

30. (New): The method of claim 28, wherein B is an organic compound.

31. (New): The method of claim 30, wherein the blocking group is selected from the group consisting of Ac-Tyr-Val-Ala-Asp--, Ac-Asp-Glu-Val-Asp--, Ac-Leu-Glu-Val-Asp--, Ac-Trp-Glu-His-Asp--, Ac-Val-Glu-Ile-Asp-- and Ac-Leu-Glu-His-Asp--.

32. (New): The method of claim 30, wherein the blocking group is selected from the group consisting of Glucose-alpha--, Glucose-beta--, Galactose-alpha--, Galactose-beta--, N-Acetyl glucosamine-- and Glucuronic acid--.

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33. (New): The method of claim 30, wherein the blocking group is selected from the group consisting of HO-Darg-Gln-Gly-Ala-Ill-Gly-Gln-Pro--, Pyr-Pro-Val-- and Benzoyl DL Arginine--.

34. (New): The method of claim 30, wherein the blocking group is selected from the group consisting of acetate--, chloroacetate-- and buyrate--.

35. (New): The method of claim 28, wherein the blocking group is an inorganic compound.

36. (New): The method of claim 35, wherein the blocking group is selected from the group consisting of HO(OO)PO-- and HO(OO)SO--.

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